Original Article

Thyroglobulin as an Evolving Biomarker of Iodine Reserve in Thyroid Dysfunction Assessment in Pregnancy

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Abstract

Background: Despite the use of routine thyroid function tests, thyroid dysfunction is often missed in pregnant women, who may have fluctuating iodine reserves and this may be associated with an increased risk of adverse maternal and fetal outcomes due to thyroid dysfunction. Therefore, thyroglobulin (Tg) as a marker of iodine reserve may be added to improve the diagnostic value of the thyroid testing panel. **Materials and Methods:** This study was a multicenter descriptive cross-sectional study with 501 healthy pregnant women, carried out over 9 months, in which blood and urine (spot) specimens were collected and analysed for serum thyroid-stimulating hormone, free thyroxine, free triiodothyronine, and Tg using enzyme-linked immunosorbent assay, and urinary iodine concentration by modified Sandell–Kolthoff reaction. **Results:** The prevalence of thyroid dysfunction in pregnant women using thyroid function tests alone was 12.4% (62) with 9.6% (48) being hypothyroid and 2.0% (10) hyperthyroid. The addition of Tg was able to identify more participants with thyroid dysfunction who were iodine deficient, and initially missed using thyroid function tests alone. This newly added biomarker to routine thyroid function tests profile increased the prevalence of thyroid disorders in this study population from 12.4% (62) to 17.6% (88) ($P < 0.01$), whereas urine iodine concentration was adequate for each trimester falling within the WHO range of 150–249 μg/l. **Conclusion:** The true prevalence of thyroid dysfunction in pregnant women in Makurdi was 17.6%. The addition of Tg had an impact on thyroid function tests by identifying more participants with iodine-related thyroid dysfunction, who were missed in the initial assessment of the thyroid. The mean urine iodine concentration was adequate, falling within the WHO range of 150–249 μg/l.

Keywords: Thyroglobulin, thyroid function tests, thyroid-stimulating hormone, thyroxine, triiodothyronine, urine iodine concentration

Introduction

Thyroid disease in pregnancy is an endocrine disorder that is usually underestimated because pregnancy may mask the effects of a thyroid dysfunction due to its hyperdynamic nature. Pregnancy is a period that places great physiological stress on both the mother and the fetus during the best of times, this may be compounded further by endocrine disorders leading to potentially adverse outcomes for maternal and fetal which could be immense.

During pregnancy, the thyroid gland increases in size by up to 10% in iodine-sufficient countries in stark contrast to 20%–40% in areas of iodine deficiency.[1,2] Thyroid disease as earlier reported affects 5% of all pregnancies.[3] Thyroid disease is second only to diabetes mellitus as the most common endocrinopathy that occurs in women during their reproductive years.[4] Thyroid disease often mimic common symptoms of pregnancy, making it challenging to identify. Thyroid-stimulating hormone (TSH) has homology with Human Chorionic Gonadotropins sharing the same alpha receptors, consequently stimulating it. Therefore, the use of thyroid function tests alone may not be entirely appropriate in identifying thyroid dysfunction in pregnancy, especially in the first trimester. To this end, the utilisation of thyroglobulin (Tg) as a biomarker has been suitable for monitoring thyroid iodine economy and its reserve.[5]

Serum Tg concentration is considered to reflect thyroid volume in both iodine-deficient and iodine-excessive settings.[6] Tg is a 660 kDa glycoprotein, produced by the thyroid follicles

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and stored within the colloid of these follicles. It is involved in the process of organification, which is a combination of the tyrosine residues of the Tg with iodine, which is a critical step in the synthesis of triiodothyronine (T3) and thyroxine (T4).

The use of Tg is not routinely incorporated when thyroid hormones are assayed in routine thyroid screens but has been well-documented as a biomarker for malignant thyroid cancer treatment monitoring. However, there has been a growing body of evidence of the use of Tg in the diagnosis of thyroid dysfunction, especially in thyroid disorders caused by iodine deficiency. Consequently, the use of Tg as an adjunct to routine thyroid testing would be able to identify patients with a thyroid disorder that may have gone unnoticed, especially in pregnant women, whose thyroid metabolism and function are already undergoing tremendous dynamic changes.

Materials and Methods

Study design and setting

This was a multicenter hospital-based descriptive cross-sectional study of pregnant women. It involved the analysis of data collected from the participants in the course of the study. The time frame for the study work was approximately 9 months (June 2019–February 2020). The recruitment of participants from the antenatal clinic and sampling of the same for serum thyroid function tests, urine iodine, laboratory analysis, and evaluation of thyroid dysfunction in pregnancy were all done during the above-stated 9 months.

The study population was drawn from Makurdi a city in North Central Nigeria. Participants were recruited from Benue State University Teaching Hospital (B. S. U. T. H), Federal Medical Center (F. M. C) Makurdi, Family Support Programme Clinic Makurdi, First Fertility Hospital Makurdi, and Foundation Hospital Makurdi. Data and sample analysis were carried out at the laboratory of the Chemical Pathology Department in B. S. U. T. H, Makurdi.

Study population

The target population was pregnant women in Makurdi attending their routine antenatal clinic visit and these participants were selected by a simple random sampling technique using a table of random numbers. They were briefed about the study, their written consent was taken and questionnaires were administered.

The participants were appropriately grouped into three trimesters, including participants with no history of thyroid dysfunction while those excluded were participants with known thyroid dysfunction, acutely or chronically ill, on specific drugs, i.e., lithium, amiodarone, antiseizure drugs, interferon-gamma, hormone replacement therapy (HRT) (estrogen), and rifampicin. Finally, each participant had their biofluids collected.

Sample size determination

The sample size required for the study was calculated using the formula:[7]

Where:

$N = Z^2pq \div (d)^2$

N = Minimum sample size

z = Standard normal deviation usually set at 1.96 (corresponding to a 95% confidence interval).

P = Prevalence of thyroid dysfunction in pregnancy from a previous study done in Northern Nigeria (Zaria) was estimated to be 5.3%

q = proportion of the target population unaffected by the condition

(i.e., $q = 1–P = 1 − 0.053 = 0.947$)

d = Tolerable margin of error, an observed difference of 5% is taken as being significant

Therefore, $n = (1.962 \times 0.053 \times 0.947) \div (0.052) = 77$

Adjusting the sample size for a 10% nonresponse rate

Nf = n/1–NR; Where Nf* = Adjustable sample size due to attrition

n = minimum sample size

NR = Nonesponse rate set at 10%

Nf = 77/1–10% = 77/1 − 0.1 = 77/0.9 = 86

Five hundred participants were to be selected as a cohort. Therefore, calculating the nonresponse rate for 500 participants using the above formula, approximately 556 participants were recruited. Thereby improving the chances of identifying those with thyroid dysfunction, enabling statistical inferences, and conclusions to be reached.

Ethical consideration

Written consents for inclusion in the study were obtained, this was done after the explanation of the study, and the procedures involved were made known to the participants. Written permissions were obtained from the heads of the department of Obstetrics/Gynaecology, Chemical Pathology of B. S. U. T. H, and The Chief Medical Directors of B. S. U. T. H Makurdi, F. M. C, and First Fertility Hospital Makurdi. Ethical clearance for the study was obtained from the Health Research Ethics Committee of each participating institute.

Data collection

The information of the participants was collected using a patient information form and questionnaires. Furthermore, the structured questionnaires comprised sociodemographic characteristics and history (past medical and drug history). The information of each participant was written on the forms that were given.

Sample collection and analysis

Nonfasting samples were collected: venous blood and spot urine, 5 ml of blood were collected by an aseptic technique using a syringe and needle into a plain vacutainer tube,

and a spot urine sample was collected in a wide-mouthed sterile urine bottle. The samples were separated using a tabletop centrifuge (StatSpin Express) at 3000 rpm for 10 min and the serum samples were pipetted and stored in cryovials at −20°C. Moreover, urine samples were analysed immediately for urine iodine concentration using the Sandell–Kolthof technique.

The batched serum samples were analysed using the ultrasensitive enzyme-linked immunosorbent assay (ELISA) technique supplied by Monobind Inc.® (AccuBind® ELISA kits, California, USA) for thyroid function test panel and Tg analysis. This analysis was carried out using an automated machine with a microstrip reader (STAT-FAX 303, USA).

Statistical analysis

Data analysis was done using Statistical Package for the Social Sciences (SPSS) version 21. (IBM, Chicago,IL, USA). Normally distributed data were expressed as mean ± standard deviation (SD), whereas nonnormally distributed data were expressed as a median.

For non-Gaussian distributed data, a comparison was performed using Mann–Whitney *U*-test and Kruskal–Wallis tests. Comparison of Gaussian distributed data was made using the unpaired Student's *t*-test and one-way analysis of variance (ANOVA), whereas Pearson's correlations determined correlations. $P < 0.05$ was considered statistically significant.

Thereafter, a test for normalcy was performed to determine if the data set followed a Gaussian or non-Gaussian distribution, determining the statistical tool to be applied. Gestational age, free T3 (FT3), free T4 (FT4), and urine iodine concentration were Gaussian in distribution, therefore, one-way ANOVA and unpaired Student's *t*-test were used while TSH and Tg were non-Gaussian in distribution as a result, Mann–Whitney *U*-test was used.

Results

The study included 556 pregnant women, who were selected by a simple random sampling technique, out of which 544 met the inclusion criteria for the study but a total of 43 participants declined to be sampled. Subsequently, a total of 501 participants were sampled for blood and urine to be analysed.

Population distribution pattern

Five hundred and one pregnant participants were studied and grouped based on their trimesters into Group I, Group II, and Group III. One hundred and three participants were in Group I, 228 in Group II, and 170 in Group III corresponding to each trimester.

Characteristics of the study population

One hundred and three participants were in Group I, 228 in Group II, and 170 in Group III corresponding to each trimester.

Table 1 depicts the gestational and chronological ages of the participants in each group. Gestational age distribution (mean ± SD) was 9 ± 2.70, 20 ± 3.70, and 33.3 ± 4.10 in Group I, Group II, and Group III, respectively, with a gestational age range of 5–40 weeks.

The chronological age of the participants ranged from 13 to 39 years with chronological age distribution (mean ± SD) of 26.40 ± 4.70, 27.00 ± 5.10, and 28.20 ± 5.20 for Group I, Group II, and Group III, respectively.

The TSH analysis provided in Table 2 shows that there was a significant statistical difference between Group I and Group II and Group I and Group III TSH values ($P < 0.01$) using the Mann–Whitney *U*-test because TSH values were nonparametric in distribution.

Table 1: Gestational age and chronological age across the trimesters

Parameters	Mean±SD		
	1st trimester (*n*=103)	2nd trimester (*n*=228)	3rd trimester (*n*=170)
Gestational age (weeks)	9±2.70	20±3.70	33.3±4.10
Chronological age (years)	26.40±4.70	27.00±5.10	28.20±5.20

SD: Standard deviation

Table 2: Median values of maternal thyroid-stimulating hormone within the trimesters using Mann-Whitney *U*-test

Parameter	Mean±SD			*P*
	1st trimester (*n*=103)	2nd trimester (*n*=228)	3rd trimester (*n*=170)	
TSH	2.76[a]	1.53[b]	3.50[c]	0.0001[a] 0.0001[b] 0.741[c]

[a]Statistically significant difference between the first and second trimesters, [b]Statistically significant difference between the second and third trimesters, [c]Statistically significant difference between the first and third trimesters. *n*: number of participants, TSH: Thyroid-stimulating hormone, SD: Standard deviation

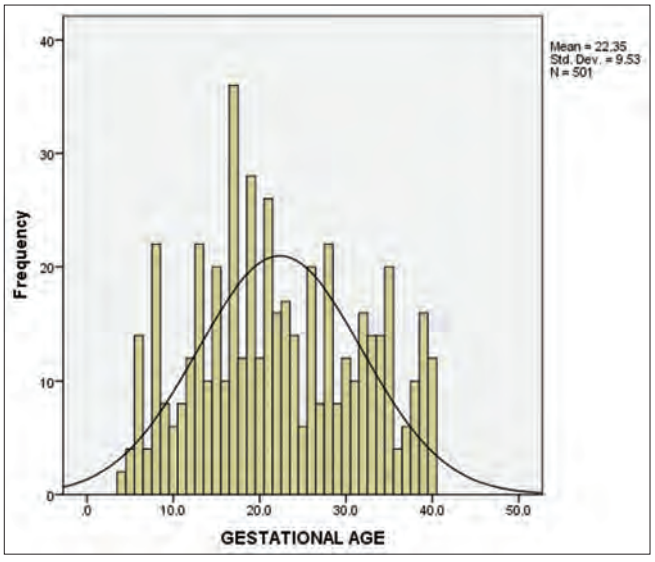

Figure 1: A histogram for gestational age

Table 3 illustrates the mean values for fT4 to be 0.75 ± 0.26 in Group I, 0.80 ± 0.30 in Group II, and 0.57 ± 0.23 in Group III. This table also illustrated the comparison of mean values for fT3, and urinary iodine concentration (UIC), the mean values for fT4 and fT3 were significant ($P < 0.01$) across all trimesters as compared to UIC which showed no statistical significance with the highest mean value of 192.02 ± 40.71 for Group I.

Figure 1 illustrates a Histogram for gestational age, which is Gaussian in distribution.

Figure 2 depicts a histogram of Thyroid stimulating hormone in a non-Gaussian distribution.

Figure 3 illustrates a correlation plot of gestational age and FT4, there was a decline in the concentration of FT4 with increasing gestational age across the three trimesters.

Table 4 stratifies the thyroid disorders using Tg assay, categorizing them into euthyroid, hyperthyroid, or hypothyroid states.

Table 5 compares the use of thyroid function tests assay only and a combination of thyroid function tests and Tg assay using the Chi-square test. TFT only identified 62 participants as

Table 3: Mean values of maternal thyroid function test parameters within the trimesters and the pattern of urine iodine concentration in each trimester using analysis of variance

Parameters	Mean±SD			*P*
	1st trimester (*n*=103)	2nd trimester (*n*=228)	3rd trimester (*n*=170)	
FT4 (pg/ml)	0.75±0.26	0.80±0.30	0.57±0.23	0.0001
FT3 (ng/dl)	1.53±0.76	2.96±1.27	1.87±0.42	<0.0001
UIC (µg/l)	192.02±40.71	185.49±32.94	186.54±35.35	0.536

SD: Standard deviation, UIC: Urinary iodine concentration, FT4: Free thyroxine, FT3: Free triiodothyronine

Table 4: Prevalence of thyroglobulin disorders in participants stratified by thyroid function tests

TFT	Normal	Abnormal	Total
Euthyroid	417	26	443
Hyperthyroid	0	0	0
Hypothyroid	38	20	58
Total	455	46	501

TFT: Thyroid function test

Table 5: Comparison of prevalence of thyroid dysfunction using thyroid function test only and thyroid function test + thyroglobulin using Chi-square test

TFT only (%)	TFT + Tg (%)	χ^2	*P*
62 (12.4)	88 (17.6)	50.3	0.00001

TFT: Thyroid function test, Tg: Thyroglobulin

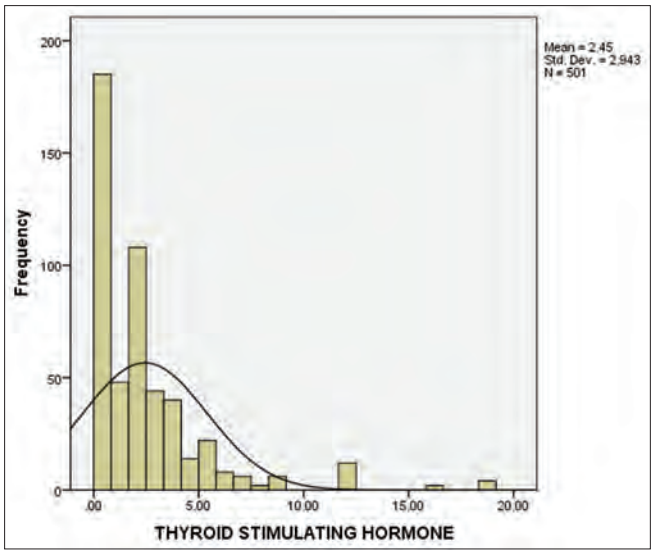

Figure 2: A histogram for TSH. TSH: Thyroid-stimulating hormone

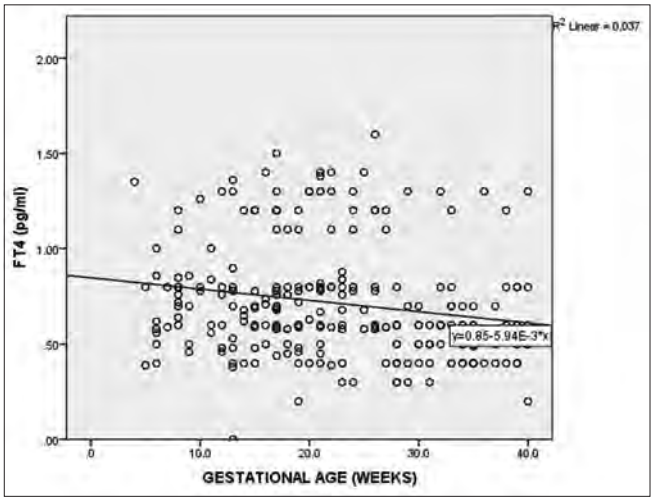

Figure 3: Correlation plot between gestational age and FT4. FT4: Free thyroxine

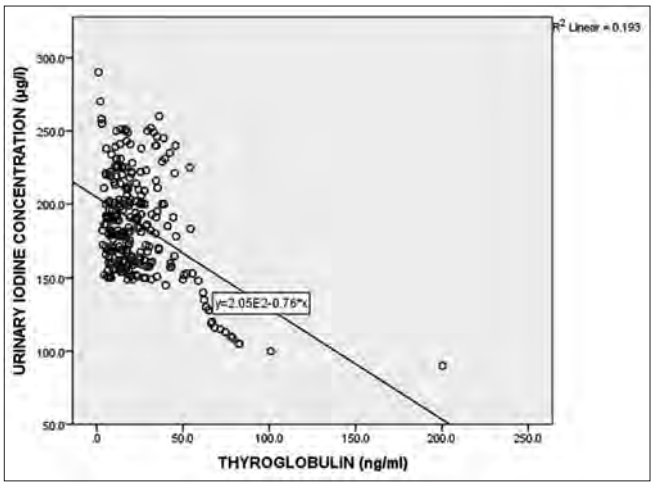

Figure 4: Correlation plot of thyroglobulin and urine iodine concentration

having thyroid dysfunction but in combination (TFT and Tg) 88 participants ($P < 0.01$) were identified, who were initially euthyroid biochemically using TFT only.

The correlation between Tg and urine iodine concentration is illustrated in Figure 4.

An inverse relationship is demonstrated between the two parameters in the graph using Pearson's correlation ($P < 0.01$). Most participant clusters were within the ranges of 150 and 249 µg/l for UIC.

Discussion

Thyroid hormones have profound variation during human life, and changes are associated with severe adverse health impacts.[8] Pregnancy which is a hyperdynamic state may affect thyroid adaptations and these adaptations may be well tolerated in an iodine-sufficient area or undergo significant changes in iodine-deficient areas.[9,10]

Therefore, the importance of identifying thyroid dysfunction cannot be overemphasized. This study was able to identify a proportion of the participants who were either hypothyroid or hyperthyroid of which 2.0% ($n = 10$) were hyperthyroid, which was similar to studies done in Zaria and Tunisia which placed hyperthyroidism in pregnancy at 2.3%[11] and 1.3%,[12] respectively. In this same study, hypothyroidism was seen in 9.6% ($n = 48$) of the participants and was significantly higher, compared to the outcomes of the studies in Zaria and Tunisia at 3% and 3.2%, respectively.

Moreover, the findings of different studies carried out on pregnant participants in the United States of America, identified hypothyroidism to be as high as 2.5%–3%[13] to 6.5%–7.5%,[14-16] which was more comparable to this study.

In addition, the variation and the unstable nature of the thyroid gland in the pregnancy state prompted the use of an additional biomarker called Tg which was considered more stable in pregnancy and a sensitive marker of iodine status and reserve as compared to other thyroid assessment parameters (TSH, T4, and T3).[17-19] With the addition of Tg assay, 26 (5.2%) more participants were identified, who were initially assessed to have normal thyroid function, using thyroid function tests only, this may be because, the changes in Tg concentration are more sensitive to the biochemical derangement of the thyroid gland before TSH, fT4, and fT3 biochemical changes can be apparent.

The use of Tg significantly increased the prevalence of thyroid dysfunction from 62 (12.4%) to 88 (17.6%) participants ($P < 0.01$). Furthermore, this study has proven that the true prevalence of thyroid dysfunction in this cohort would have been underestimated using thyroid function tests only. This finding agrees with a recent study in the United Kingdom which proved Tg to be a promising functional biomarker of iodine status during pregnancy.[20] Although, this study reported the mean UIC in spot urine, to be within the iodine sufficiency range of 150–249 µg/l in pregnant women as recommended by the WHO,[21,22] some participants still fell outside the acceptable range of urine iodine concentration stated for pregnant women. The findings of this study appeared to be in the middle of the global picture, a situation that is not surprising given that Nigeria is an iodine-sufficient nation[23] due to the launch of the universal salt iodization programme in iodine-insufficient regions in 2008.[24]

The correlation between urine iodine concentration and Tg was an inverse relationship. This implies that a lower UIC would be associated with a higher level of Tg. This was similar to a study done in the Benin republic to measure iodine supply in school children.[16]

Conclusion

The true prevalence of thyroid dysfunction in pregnant women in Makurdi was 17.6%. The addition of Tg had an impact on thyroid function tests by identifying more participants with iodine-related thyroid dysfunction, who were missed in the initial assessment of the thyroid. The mean UIC was adequate, falling within the WHO range of 150–249 µg/l. The future of identifying iodine-associated thyroid dysfunction lies in the use of Tg as a marker of thyroid iodine status and reserve.

Recommendation

The addition of Tg assay to routine thyroid function testing panel in detecting thyroid dysfunction due to iodine insufficiency in pregnancy is more reliable, especially in iodine-insufficient regions. The stability of Tg in pregnancy over other routine thyroid testing parameters is recommended because of the effect of pregnancy on baseline hormones of the thyroid.

Limitations of the study

Twenty-four-hour urine would have been ideal for iodine–creatinine ratio but this was difficult due to patient cooperation, the cumbersome process of a 24-h urine collection, and storage as a result a spot urine sample was used instead.

Author contributions

All authors have accepted responsibility for the entire content of this manuscript and approved its submission.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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